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**Agmatine-containing poly(amidoamine)s as novel class of antiviral macromolecules:
structural properties and in vitro evaluation of infectivity inhibition**

Manuela Donalisio¹, Elisabetta Ranucci², Valeria Cagno¹, Andrea Civra¹, Amedea Manfredi²,
Roberta Cavalli³, Paolo Ferruti², David Lembo^{1*}

¹Dipartimento di Scienze Cliniche e Biologiche, Università degli Studi di Torino, 10043 Orbassano, Torino, Italy;

²Dipartimento di Chimica Organica e Industriale, Università degli Studi di Milano, 20133 Milano, Italy; ³Dipartimento
di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, 10125 Torino, Italy

Running title: Antiviral properties of PAAs

* Corresponding author: Prof. David Lembo
Department of Clinical and Biological Sciences
University of Torino,
Regione Gonzole, 10
10043, Orbassano, Turino, Italy
Phone: +39 011 6705484
Fax: +39 011 2365484
E-mail: david.lembo@unito.it

36 **ABSTRACT**

37

38 Poly(amidoamine)s (PAAs) are multifunctional *tert*-amine polymers endowed with high structural
39 versatility. Here we report on the screening of a minilibrary of PAAs against a panel of viruses. The
40 PAA AGMA1 showed antiviral activity against herpes simplex virus, human cytomegalovirus,
41 human papillomavirus-16, and respiratory syncytial virus, but not against human rotavirus and
42 vesicular stomatitis virus. The results suggest the contribution of both polycationic nature and side
43 guanidine groups in imparting antiviral activity.

44

45 The development of antiviral molecules usually focuses on either preventing virus entry into the
46 host cell or inhibiting virus replication following host infection. The first strategy may be based on
47 antiviral polyanionic polymers capable of competitively blocking the interaction between viral
48 proteins and cell surface heparan sulfate proteoglycans (HSPGs), which are exploited as attachment
49 receptors by many viruses (1, 2, 3, 4). Notwithstanding the large number of studies demonstrating
50 their efficacy in preclinical models, polyanionic polymers somehow failed in clinical trials (5).
51 Unlike polyanions, polycationic polymers have been less investigated as antiviral compounds. In
52 principle, polycations could act as antivirals by electrostatically interacting either with the
53 negatively charged cell membrane or with the envelope of lipid-enveloped viruses, thus preventing
54 virus adsorption onto cell surfaces, or directly inactivating the virus particle. In this context, it was
55 shown that the cationic poly(acrylic ester) Eudragit E100, endowed with a membrane-destabilizing
56 activity, exerts antiviral activity against a panel of lipid-enveloped viruses (6, 7). Another study
57 demonstrated that polyethylenimine, a cationic polymer able to condense DNA and mediate gene
58 transfer into mammalian cells, inhibits infection by human cytomegalovirus (HCMV) and human
59 papillomavirus (HPV), a lipid-enveloped virus and a non-enveloped virus respectively (8).
60 Poly(amidoamine)s (PAAs) are multifunctional *tert*-amine polymers endowed with high structural
61 versatility, obtained by Michael polyaddition of amines and bis-acrylamides (9). The repeating units
62 of PAAs can be designed to be reminiscent of peptides. For instance, an amphoteric, prevalingly
63 cationic PAA named AGMA1 is a polymer mimic of *arg-gly-glu* peptide (RGD) (10, 11).
64 In the search of new antiviral compounds, a minilibrary of PAAs was screened against a panel of
65 seven viruses, namely herpes simplex virus type 1 and 2 (HSV-1, HSV-2), HCMV, HPV-16,
66 human respiratory syncytial virus (RSV), human rotavirus (HRV) and vesicular stomatitis virus
67 (VSV) chosen as representatives of different virus characteristics such as presence or absence of
68 lipid envelope, nature of genome (DNA or RNA) and HSPG dependency for virus attachment (12,
69 13, 14, 15, 16).
70 The minilibrary included three water-soluble PAAs, ISA1, ISA23 and AGMA1, whose structures

71 are reported in Figure 1. The copolymeric ISA1 (17), containing two randomly distributed repeating
72 units present in equal amounts, and the homopolymeric ISA 23 (17, 18) and AGMA1 (19) were
73 prepared as previously reported. AGMA1 fractions with different average molecular weights were
74 obtained by ultrafiltration against water using membranes with different nominal molecular weight
75 cut off, as previously described (20). ISA1, is weakly cationic, but ISA23 and AGMA1 are
76 amphoteric, with isoelectric points ~ 5.2 and ~ 10.3 . As reported in Table 1, at pH 7.4, these PAAs
77 have, respectively, $+0.55$, -0.55 and $+0.55$ average charges per unit. For ISA1, the reported value
78 corresponds to the ionization degree of its *ter*-amine groups, no other ionizable groups being
79 present. For ISA23 and AGMA1, the reported figures correspond to the excess negative over
80 positive charges and vice-versa, that is, respectively, $-1+0.45$ and $-1+1.55$ per unit. Thus, at pH 7.4
81 the overall cationic charge of ISA1 and AGMA1 is superficially similar, but a deeper insight
82 reveals that their real charge distribution is different.

83 Antiviral assays were performed by infecting cell monolayers in presence of serial dilutions of
84 compounds for 2 hours at 37°C to generate dose-response curves and a selectivity profile of the
85 PAAs antiviral spectrum. The inocula were subsequently washed out and replaced with culture
86 medium containing the same concentration of compounds. The effect on HSV and VSV infections
87 was evaluated by a standard plaque reduction assay on pre-seeded Vero cells in 24-well plates ($10 \times$
88 10^4 cells) infecting with 300 pfu/well of clinical isolates of HSV-1 and HSV-2 (21), and Vesicular
89 stomatitis virus (VSV) serotype Indiana; after incubation for 24 hours (HSV-2 and VSV) or 48
90 hours (HSV-1) at 37°C in 5% CO_2 , cells were fixed and stained with 0.1% crystal violet in 20%
91 ethanol and viral plaques were counted. The mean plaque count for each drug concentration was
92 expressed as a percentage of the mean plaque count of the control.

93 In HCMV and RSV inhibition assays, infected cells were fixed and subjected to virus-specific
94 immunostaining as described previously (22, 23). In these assays, cells were pre-seeded at a density
95 of 6×10^3 /well in 96-well plates. Hep-2 cells were infected with RSV strain A2 (60 pfu/well)

96 whereas HELF cells with HCMV strain AD169 (24 pfu/well). Three days (RSV) or five days
97 (HCMV) post-infection viral immunostained plaques were microscopically counted.

98 HPV inhibition assays were performed on preplated 293TT cells (2×10^4 /well in 96-well plates)
99 using HPV-16 SEAP (secreted alkaline phosphatase) pseudoviruses (PsV) at the final concentration
100 of 1 ng/ml L1; three days post infection the SEAP content in the clarified supernatant was
101 determined as previously described (24). Plasmids used for PsV production were kindly provided
102 by J. Schiller (NCI, Bethesda USA). Antiviral assays for rotavirus were carried out on preplated
103 MA104 cells (1×10^4 /well in 96-well plates) using human rotavirus strain Wa (200 pfu/well). After
104 16 hours, viral foci were determined by indirect immunostaining (24).

105 The end-points of the assays were the effective compound concentration that reduced the viral
106 plaque/focus formation or SEAP activity by 50% (EC_{50}) in comparison to the untreated control. F
107 test was used to compare $LogEC_{50}$ s and two-way analysis of variance to analyze the significance
108 between percentages of infection at the same doses of different compounds not able to generate
109 EC_{50} s. Values of $p < 0.05$ were considered statistically significant. The EC_{50} values and all
110 statistical analyses were calculated by using the program PRISM 4 (GraphPad Software, San Diego,
111 California, U.S.A.). Cell viability assays were performed on cells pre-seeded in 96-well plates
112 under identical culture conditions of antiviral assays (i.e. cell density and time of incubation with
113 compounds) using CellTiter 96 Proliferation Assay Kit (Promega, Madison, WI, USA). The 50%
114 cytotoxic concentrations (CC_{50}) were determined using Prism software and the selectivity index
115 (SI) was calculated by dividing the CC_{50} by the EC_{50} (21). All data were generated from duplicate
116 wells in at least three independent experiments. Heparin was included in the study as a positive
117 control being a known inhibitor of HSPG-dependent viruses (e.g. HSV-1, HSV-2, HCMV, RSV
118 and HPV-16) (25, 26, 27, 28). As expected, heparin blocked infection by HSPG-dependent viruses
119 but not that by VSV and HRV which are not dependent on HSPG (Table 2).

120 Data reported in Table 2 prompt the following observations. The PAA antiviral effect was not a
121 consequence of cytotoxicity, since none of the screened compounds significantly reduced cell

122 viability at any concentration tested (i.e. up to 300 µg/ml); therefore their CC₅₀ values may be
123 considered to be higher than 300 µg/mL in all the cell lines tested.

124 Polydisperse AGMA1 strongly inhibited infections by HSV-1, HSV-2, HCMV and HPV-16,
125 generating dose response-curves with EC₅₀ values of 3.04, 5.34, 0.76, 0.54 µg/mL respectively.
126 Interestingly, AGMA1 was significantly more active than heparin against HSV-1 and HPV-16
127 infections, whereas was as active as heparin against HCMV infection ($p < 0.05$). By contrast,
128 polydisperse AGMA1 was inactive against RSV, HRV and VSV.

129 To evaluate the influence of molecular weight on antiviral potency, three additional linear AGMA1
130 fractions were prepared, namely AGMA1₄ (\overline{M}_n 4500), AGMA1₇ (\overline{M}_n 7800) and AGMA1₂₀ (\overline{M}_n
131 20500) (Table 1). As depicted in Table 2, fractions with lower and higher molecular weights than
132 polydisperse AGMA1 (\overline{M}_n 10100) maintained inhibitory activity against HSV-1, HSV-2, HCMV
133 and HPV-16 although to different extents. AGMA1₄ showed a stronger anti-HSV-1 activity than
134 that of heparin and all fractions were more active than heparin against HPV-16 infection ($p < 0.05$).
135 No statistically significant differences were observed between EC₅₀ of heparin and EC₅₀s of
136 AGMA1₄ against HSV-2 and HCMV infections and between EC₅₀ of heparin and EC₅₀ of
137 AGMA1₂₀ against HSV-1 infection.

138 Unlike polydisperse AGMA1, AGMA1₄, AGMA1₇ and AGMA1₂₀ were active also against RSV
139 with EC₅₀ values of 8.87, 7.44, and 1.37 µg/mL respectively. Both polydisperse AGMA1 and
140 AGMA1 fractions failed to display any significant inhibitory effect against HRV and VSV. The
141 antiviral activity of AGMA1 seems not to be dependent on its molecular weight for HSV-1, HSV-2,
142 HCMV and HPV-16, instead there is a clear relationship between AGMA1 fractions' size and anti-
143 RSV potency. Explaining why polydisperse AGMA1 did not exert a detectable anti-RSV activity,
144 while all of the size fractions did, demands further investigation.

145 Polymers do not consist of a single molecular specie, but rather of families of homologous species
146 differing for the number of repeating units. Therefore, it is considered inappropriate to adopt the
147 molar concept describing their properties. Nevertheless, to compare activity across compounds,

Table 3 shows the EC₅₀ values of AGMA1 fractions and heparin expressed in terms of molarity instead of µg/ml, considering the average molecular weight reported in Table 1. It was not possible to convert the average molecular mass of polydisperse AGMA1 in terms of molar equivalents since its molecular mass is not univocally defined. Interestingly, the relationship between AGMA1 fractions' size and anti-RSV potency, reported in text when data were expressed in terms of µg/ml, is preserved. Furthermore, AGMA1₇ and AGMA1₂₀ preserved a higher anti-HPV-16 activity than that of heparin ($p < 0.05$). By contrast, the antiviral activity of AGMA1₄ in terms of molarity is lower than that in terms of µg/ml: its activity is similar to that of heparin against HSV-1 and HPV-16 infections and is lower to that of heparin against HSV-2 and HCMV ($p < 0.05$). This behavior might be ascribed to a more rigidity of the polymer with the lowest molecular weight. Being all the polymers polyelectrolytes, it is necessary to take into account that the charge density markedly affect the dynamic rheological properties, the flexibility and the chain entanglements. Increased polymer charge density results in intermolecular electrostatic repulsion and increased polymer solubility.

Next, to investigate whether the activity of AGMA1 was specifically due to the structure of its repeating unit, the antiviral activity of ISA1 and ISA23 was assessed. Overall, whilst AGMA1 was active against HSV-1, HSV-2, HCMV, RSV and HPV-16 infection, ISA1 was active only against HCMV and RSV with a lower activity than that of heparin and was as active as heparin against HPV-16 ($p < 0.05$). ISA23 was inactive in all cases. At pH 7.4, both AGMA1 and ISA1 are positively charged, whereas ISA23 is negatively charged. It is known that polycationic polymers establish ionic interactions with the cell surface HSPG (29, 30), a feature that may impart antiviral activity to these compounds. This feature, along with the finding that the active PAAs have the same antiviral activity spectrum as heparin, supports the hypothesis that PAAs may exert their antiviral action, at least in part, by interacting with HSPG thus preventing virus attachment. However, notwithstanding AGMA1 and ISA1 carry the same density of positive charges, i.e. +0.55, AGMA1 showed a greater activity for HSV-1, HSV-2 and HPV-16. This could be due to the

174 different real charge distribution on the macromolecules and to its side guanidine groups reinforcing
175 membrane interactions, according to their well-known chaotropic properties (31). By contrast, the
176 guanidine side group does not seem to be necessary for the anti-HCMV activity. Furthermore, a
177 different chain entanglement might explain the different activity of AGMA1 in respect to RSV.
178 Overall these results provide a starting point to tailor a macromolecule with enhanced antiviral
179 activity against a selected virus. Future work will be focused on narrowing the molecular mass
180 distribution of PAA samples to assist in preclinical development.
181 Studies are ongoing to elucidate the mechanism of action the active PAAs and their antiviral
182 potential and biocompatibility profile in preclinical models.

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200 REFERENCES

- 201 1. **Spillmann D.** 2001. Heparan sulfate: anchor for viral intruders? *Biochimie.* **83**:811-817.
- 202 2. **Liu J, Thorp SC.** 2002. Cell surface heparan sulfate and its roles in assisting viral
203 infections. *Med Res Rev.* **22**:1-25.
- 204 3. **Tiwari V, Maus E, Sgar IM, Ramsey KH, Shukla D.** 2012. Role of heparan sulfate in
205 sexually transmitted infections. *Glycobiology* **22**:1402-1412.
- 206 4. **Rusnati M, Vicenzi E, Donalisio M, Oreste P, Landolfo S, Lembo D.** 2009. Sulfated K5
207 *Escherichia coli* polysaccharide derivatives: A novel class of candidate antiviral
208 microbicides. *Pharmacol Ther.* **123**:310-322.
- 209 5. **Pirrone V, Wigdahl B, Krebs FC.** 2011. The rise and fall of polyanionic inhibitors of the
210 human immunodeficiency virus type 1. *Antiviral Res.* **90**:168-182.
- 211 6. **Alasino RV, Ausar SF, Bianco ID, Castagna LF, Contigiani M, Beltramo DM.** 2005.
212 Amphipathic and membrane-destabilizing properties of the cationic acrylate polymer
213 Eudragit E100. *Macromol Biosci.* **5**:207-213.
- 214 7. **Alasino RV, Bianco ID, Vitali MS, Zarzur JA, Beltramo DM.** 2007. Characterization of
215 the inhibition of enveloped virus infectivity by the cationic acrylate polymer eudragit E100.
216 *Macromol Biosci.* **7**(9-10):1132-1138.
- 217 8. **Spoden GA, Besold K, Krauter S, Plachter B, Hanik N, Kilbinger AF, Lambert C,
218 Florin L.** 2012. Polyethylenimine is a strong inhibitor of human papillomavirus and
219 cytomegalovirus infection. *Antimicrob Agents Chemother.* **56**(1):75-82.
- 220 9. **Ferruti P.** 2013. Poly(amidoamine)s: Past, Present, and Perspectives. *Journal of polymer
221 science, part A: Polymer Chemistry* **51**:2319–2353.
- 222 10. **Ferruti P, Franchini J, Bencini M, Ranucci E, Zara GP, Serpe L, Primo L, Cavalli R.**
223 2007. Prevalingly cationic agmatine-based amphoteric polyamidoamine as a nontoxic,
224 nonhemolytic, and "stealthlike" DNA complexing agent and transfection promoter.
225 *Biomacromolecules* **8**(5):1498-1504.

- 226 11. **Cavalli R, Bisazza A, Sessa R, Primo L, Fenili F, Manfredi A, Ranucci E, Ferruti P.**
227 2010. Amphoteric agmatine containing polyamidoamines as carriers for plasmid DNA in
228 vitro and in vivo delivery. *Biomacromolecules* **11**(10):2667-2674.
- 229 12. **Roizman B, Knipe MM, Whitley RJ.** 2007. p. 2501-2601. Herpes Simplex Viruses. *In* B.
230 N. Fields, D. M. Knipe, and P. M. Howley (ed.), *Fields Virology*, Lippincott-Raven,
231 Philadelphia, PA
- 232 13. **Lowy DR, Howley PM.** 2001. Papillomaviruses. p. 2231-2264. *In* Fields BN, Knipe DM,
233 Howley PM (ed.) *Fields Virology*, Lippincott-Raven, Philadelphia, PA.
- 234 14. **Collins PL, Crowe JE Jr.** 2007. Respiratory syncytial virus and Metaneumovirus. p 1601–
235 1646. *In* Knipe DM, Howley PM (ed.) *Fields Virology*, 5th edition. Lippincott Williams and
236 Wilkins, Philadelphia, PA
- 237 15. **Landolfo S, Gariglio M, Gribaudo G, Lembo D.** 2003. The human cytomegalovirus.
238 *Pharmacol Ther.* **98**(3):269-297.
- 239 16. **Cox E, Christenson JC.** 2012. Rotavirus. *Pediatr Rev.* **33**(10):439-445.
- 240 17. **Richardson S, Ferruti P, Duncan R.** 1999. Poly(amidoamine)s as potential endosomolytic
241 polymers: evaluation in vitro and body distribution in normal and tumour-bearing animals. *J*
242 *Drug Target.* **6**(6):391-404.
- 243 18. **Ferruti P, Manzoni S, Richardson SCW, Duncan R, Patrick NG, Mendichi R, Casolaro**
244 **M.** 2000. Amphoteric linear poly(amido-amine)s as endosomolytic polymers: Correlation
245 between physicochemical and biological properties. *Macromolecules* **33**:7793-7800.
- 246 19. **Ferruti P, Franchini J, Bencini M, Ranucci E, Zara GP, Serpe L, Primo L, Cavalli R.**
247 2007. Prevailingly cationic agmatine-based amphoteric polyamidoamine as a nontoxic,
248 nonhemolytic, and "stealthlike" DNA complexing agent and transfection promoter.
249 *Biomacromolecules* **8**(5):1498-1504.

- 250 20. **Cavalli R, Bisazza A, Sessa R, Primo L, Fenili F, Manfredi A, Ranucci E, Ferruti P.**
251 2010. Amphoteric agmatine containing polyamidoamines as carriers for plasmid DNA in
252 vitro and in vivo delivery. *Biomacromolecules* **11**(10):2667-2674.
- 253 21. **Donalisio M, Nana HM, Ngono Ngane RA, Gatsing D, Tiabou Tchinda A, Rovito R,**
254 **Cagno V, Cagliero C, Boyom FF, Rubiolo P, Bicchi C, Lembo D.** 2013. In vitro anti-
255 Herpes simplex virus activity of crude extract of the roots of *Nauclea latifolia* Smith
256 (Rubiaceae). *BMC Complement Altern Med.* **13**(1):266.
- 257 22. **Donalisio M, Rusnati M, Cagno V, Civra A, Bugatti A, Giuliani A, Pirri G, Volante M,**
258 **Papotti M, Landolfo S, Lembo D.** 2012. Inhibition of Human Respiratory Syncytial Virus
259 Infectivity by a Dendrimeric Heparan Sulfate-Binding Peptide, *Antimicrob. Agents.*
260 *Chemother.* **56**: 5278-5288.
- 261 23. **Funaro A, Gribaudo G, Luganini A, Ortolan E, Lo Buono N, Vicenzi E, Cassetta L,**
262 **Landolfo S, Buick R, Falciola L, Murphy M, Garotta G, Malavasi F.** 2008. Generation
263 of potent neutralizing human monoclonal antibodies against cytomegalovirus infection from
264 immune B cells. *BMC Biotechnol.* **8**:85.
- 265 24. **Donalisio M, Rusnati M, Civra A, Bugatti A, Allemand D, Pirri G, Giuliani A,**
266 **Landolfo S, Lembo D.** 2010. Identification of a dendrimeric heparan sulfate-binding
267 peptide that inhibits infectivity of genital types of human papillomaviruses. *Antimicrob*
268 *Agents Chemother.* **54**(10):4290-4299.
- 269 25. **WuDunn D, Spear PG.** 1989. Initial interaction of herpes simplex virus with cells is
270 binding to heparan sulphate. *J. Virol.* **63**:52- 58.
- 271 26. **Kari B, Gehrz R.** 1992. A human cytomegalovirus glycoprotein complex designated gC-II
272 is a major heparin-binding component of the envelope. *J Virol.* **66**(3):1761-1764.
- 273 27. **Hallak LK, Spillmann D, Collins PL, Peeples ME.** 2000. Glycosaminoglycan sulfation
274 requirements for respiratory syncytial virus infection. *J.Virol.* **74**:10508 –10513.
- 275 28. **Joyce JG, Tung J-S, Przysiecki CT, Cook JC, Lehman ED, Sands JA, Jansen KU,**

- 276 **Keller PM.** 1999. The L1 major capsid protein of human papillomavirus Type 11
277 recombinant virus-like particles interacts with heparin and cell-surface glycosaminoglycans
278 on human keratinocytes. *J. Biol. Chem.* **274**: 5810- 5822.
- 279 29. **Poon GM, Gariépy J.** 2007. Cell-surface proteoglycans as molecular portals for cationic
280 peptide and polymer entry into cells. *Biochem Soc Trans.* **35**(4):788-793.
- 281 30. **Mislick KA, Baldeschwieler JD.** 1996. Evidence for the role of proteoglycans in cation-
282 mediated gene transfer. *Proc Natl Acad Sci U S A.* **93**(22):12349-12354.
- 283 31. **Myers JK, Pace CN, and Scholtz JM.** 1995. Denaturant m values and heat capacity
284 changes: relation to changes in accessible surface areas of protein unfolding. *Protein Sci.* **4**:
285 2138–2148.

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289 **FIGURE LEGENDS**

290 **Figure 1.** Chemical structure of AGMA1, ISA1 and ISA 23 repeating units

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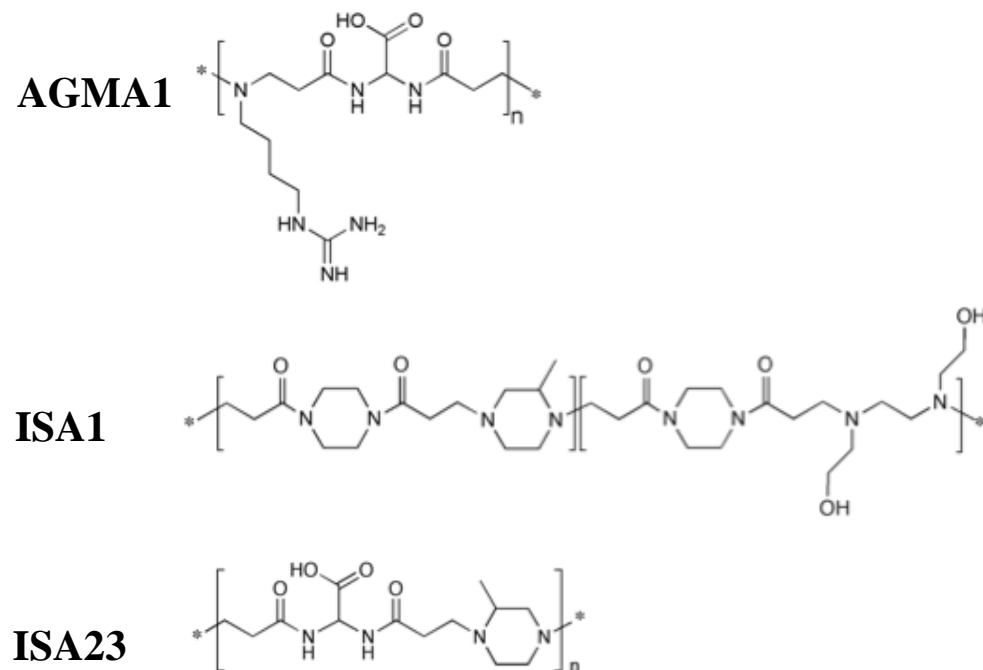


Figure 1. Chemical structure of AGMA1, ISA1 and ISA 23 repeating units

Table 1. Physico-chemical characteristics of PAAs

Polymer	\overline{M}_n	Net average charge per unit at pH 7.4	Average negative charges per unit at pH 7.4	Average positive charges per unit at pH 7.4
Polydisperse AGMA1	10100	+ 0.55	-1.00	+ 1.55
AGMA1 ₄	4500	+ 0.55	-1.00	+ 1.55
AGMA1 ₇	7800	+ 0.55	-1.00	+ 1.55
AGMA1 ₂₀	20500	+ 0.55	-1.00	+ 1.55
ISA1	13600	+ 0.55	0.0	+ 0.55
ISA23	16500	- 0.55	-1.00	+ 0.45

\overline{M}_n = number average molecular weight. $\overline{M}_n = \frac{\sum_{i=1}^n N_i \times M_i}{\sum_{i=1}^n N_i}$, where N_i = number of macromolecules

containing i repeating units, and M_i = weight of macromolecules containing i repeating units.

Table 2. Antiviral activities of PAAs and heparin

Compounds	virus	EC ₅₀ (µg/ml) ^a (95% C.I.) ^b	CC ₅₀ (µg/ml) ^c	SI
AGMA1	HSV-1	3.04 (1.75 - 5.28)	> 300	> 98.7
	HSV-2	5.34 (1.85 - 15.4)	> 300	> 56.2
	HCMV	0.76 (0.40 - 1.47)	> 300	> 395
	HPV-16	0.54 (0.53 - 0.55)	> 300	> 556
	RSV	> 100	> 300	n.a. ^d
	HRV	> 100	> 300	n.a.
	VSV	> 100	> 300	n.a.
AGMA1₄	HSV-1	1.93 (1.43 - 2.61)	> 300	> 155
	HSV-2	1.35 (0.57 - 3.17)	> 300	> 222
	HCMV	0.39 (0.11 - 1.30)	> 300	> 769
	HPV-16	0.92 (0.53 - 1.58)	> 300	> 326
	RSV	8.87 (6.51 - 12.1)	> 300	> 33.8
	HRV	> 100	> 300	n.a.
	VSV	> 100	> 300	n.a.
AGMA1₇	HSV-1	17.0 (11.4 - 25.4)	> 300	> 17.6
	HSV-2	4.80 (3.13 - 7.35)	> 300	> 62.5
	HCMV	4.45 (3.28 - 5.90)	> 300	> 67.4
	HPV-16	0.79 (0.44 - 1.44)	> 300	> 380
	RSV	7.44 (3.11 - 17.8)	> 300	> 40.3
	HRV	> 100	> 300	n.a.
	VSV	> 100	> 300	n.a.
AGMA1₂₀	HSV-1	5.10 (3.21 - 8.10)	> 300	> 58.8
	HSV-2	2.82 (1.72 - 4.64)	> 300	> 106
	HCMV	4.14 (2.50 - 6.86)	> 300	> 72.5
	HPV-16	0.72 (0.50 - 1.06)	> 300	> 417
	RSV	1.37 (1.11 - 1.68)	> 300	> 219
	HRV	> 100	> 300	n.a.
	VSV	> 100	> 300	n.a.
ISA1	HSV-1	> 100	> 300	n.a.
	HSV-2	> 100	> 300	n.a.
	HCMV	1.26 (0.79 - 2.00)	> 300	> 238
	HPV-16	3.55 (1.97 - 6.40)	> 300	> 84.5
	RSV	9.54 (5.51 - 16.5)	> 300	> 31.4
	HRV	> 100	> 300	n.a.
	VSV	> 100	> 300	n.a.
ISA23	HSV-1	> 100	> 300	n.a.
	HSV-2	> 100	> 300	n.a.
	HCMV	> 100	> 300	n.a.
	HPV-16	> 100	> 300	n.a.
	RSV	> 100	> 300	n.a.
	HRV	> 100	> 300	n.a.
	VSV	> 100	> 300	n.a.
Heparin	HSV-1	5.22 (4.22 - 6.45)	> 300	> 57.5
	HSV-2	0.67 (0.39 - 1.18)	> 300	> 448
	HCMV	0.38 (0.24 - 0.64)	> 300	> 789
	HPV-16	2.88 (1.81 - 4.57)	> 300	> 104

RSV	0.05 (0.04 – 0.06)	> 300	> 6000
HRV	> 100	> 300	n.a.
VSV	> 100	> 300	n.a.

^a EC₅₀: 50% effective concentration

^b 95% CI: 95% confidence interval

^c CC₅₀: 50% cytotoxic concentration

^d n.a.: not assessable

Table 3. Antiviral activities of Poly(amidoamine)s expressed in terms of approximate molar values

Compounds	virus	EC ₅₀ (μM) ^a (95% C.I.) ^b	CC ₅₀ (μM) ^c
AGMA1₄	HSV-1	0.43 (0.30 – 0.61)	> 66.67
	HSV-2	0.30 (0.11 – 0.80)	> 66.67
	HCMV	0.33 (0.09 – 1.27)	> 66.67
	HPV-16	0.20 (0.12 – 0.33)	> 66.67
	RSV	1.97 (1.44 – 2.69)	> 66.67
	HRV	> 22.22	> 66.67
	VSV	> 22.22	> 66.67
AGMA1₇	HSV-1	2.18 (0.65 – 7.33)	> 38.46
	HSV-2	0.61 (0.38 – 1.00)	> 38.46
	HCMV	0.56 (0.41 – 0.76)	> 38.46
	HPV-16	0.10 (0.06 – 0.18)	> 38.46
	RSV	0.95 (0.40 – 2.28)	> 38.46
	HRV	> 12.82	> 38.46
	VSV	> 12.82	> 38.46
AGMA1₂₀	HSV-1	0.25 (0.16 – 0.40)	> 14.63
	HSV-2	0.14 (0.08 – 0.23)	> 14.63
	HCMV	0.20 (0.12 – 0.33)	> 14.63
	HPV-16	0.04 (0.02 – 0.51)	> 14.63
	RSV	0.07 (0.05 – 0.08)	> 14.63
	HRV	> 4.87	> 14.63
	VSV	> 4.87	> 14.63
Heparin	HSV-1	0.38 (0.30 – 0.49)	> 21.90
	HSV-2	0.04 (0.03 – 0.07)	> 21.90
	HCMV	0.03 (0.02 – 0.05)	> 21.90
	HPV-16	0.21 (0.13 – 0.36)	> 21.90
	RSV	0.01 (0.00 – 0.01)	> 21.90
	HRV	> 7.30	> 21.90

^a EC₅₀: 50% effective concentration^b 95% CI: 95% confidence interval^c CC₅₀: 50% cytotoxic concentration